BIOSYSTEMATICS OF TETRAPLOID *EUCHARIS* (AMARYLLIDACEAE)[1]

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ABSTRACT

Eucharis is a genus of 16 species of petiolate-leaved, neotropical Amaryllidaceae restricted to rainforest understory from Guatemala to Bolivia. The two northernmost species, *E. bonplandii* and *E. bouchei,* are the only two tetraploid ($2n = 92$) species so far known in the genus. *Eucharis bonplandii* is known from only a few localities in central Colombia. *Eucharis bouchei,* restricted to Central America, is particularly polymorphic and three varieties are recognized largely on the basis of staminal cup morphology. Data from phenetic, chromosomal, and preliminary electrophoretic analyses are presented for both tetraploid species. On the basis of 17 floral characters, the three varieties of *E. bouchei* do not resolve into discrete phenetic groups. The tetraploid representatives of *Eucharis* exhibit a wide degree of karyotypic heteromorphism. *Eucharis bouchei* var. *dressleri* is an unstable tetraploid. Electrophoretic banding patterns for aspartate-amino-transferase exhibit additive effects of polyploidy in some individuals. Isozyme phenotypes of *Eucharis bouchei* var. *bouchei* are quite variable and cladistic analysis of the isozyme data suggests that this variety may be polyphyletic. *Eucharis bonplandii* and *E. bouchei* may be monophyletic sister taxa and the remnants of a once more widespread tetraploid complex. The entry of *Eucharis* into Central America was probably a geologically recent event. It is hypothesized that *E. bouchei* has been steadily migrating away from the Colombian border. *Eucharis bouchei* is a semi-species complex of geographically isolated populations in the process of morphological diversification. The evolution of *E. bouchei* var. *dressleri* may be a sympatric speciation event. Founder effects, rapid chromosomal change, and geographical isolation are considered the most important factors in the evolution of the *E. bouchei* complex. Tetraploidy and attendant increased levels of heterozygosity may have been important in facilitating the migration of *Eucharis* across the Isthmus of Panama.

The genus *Eucharis* Planchon & Linden [Amaryllidaceae "infrafamily" Pancratioidinae sensu Traub (1957, 1963)] consists of 16 species of rare, petiolate-leaved, bulbous geophytes inhabiting the understory of primary rainforest from Guatemala to Bolivia (Meerow, 1986). Most of the species are found in the western Amazon basin and adjacent slopes of the eastern Andes. *Eucharis bouchei* Woodson & Allen is a highly polymorphic tetraploid ($2n = 92$) complex of Central America (Fig. 1). The species is concentrated in Panama (Fig. 2), but has also been recorded from Costa Rica and Guatemala. *Eucharis bouchei* is the northernmost species of *Eucharis* and the only one found north of the Darién Gap. It is also the most variable species in the genus, in characteristics that elsewhere justify specific delimitation. Patterns of variation in floral size and tube and limb habit form a complete mosaic throughout the range of *E. bouchei,* showing little or no geographic consistency.

In my recent monograph of *Eucharis* (Meerow, 1986), three varieties are recognized chiefly on the basis of staminal cup morphology (Fig. 3): *E. bouchei* var. *bouchei,* var. *darienensis* Meerow, and var. *dressleri* Meerow.

Variety *bouchei,* most common around El Valle de Antón in Coclé Province (Fig. 2), is recognized by its largely edentate staminal cup in which the trapezoidal free filament is not markedly constricted distally into a narrow subulate portion (Figs. 1C, 3A, B). It is the most variable of the three varieties, both in flower size and staminal cup morphology. The staminal cup of variety

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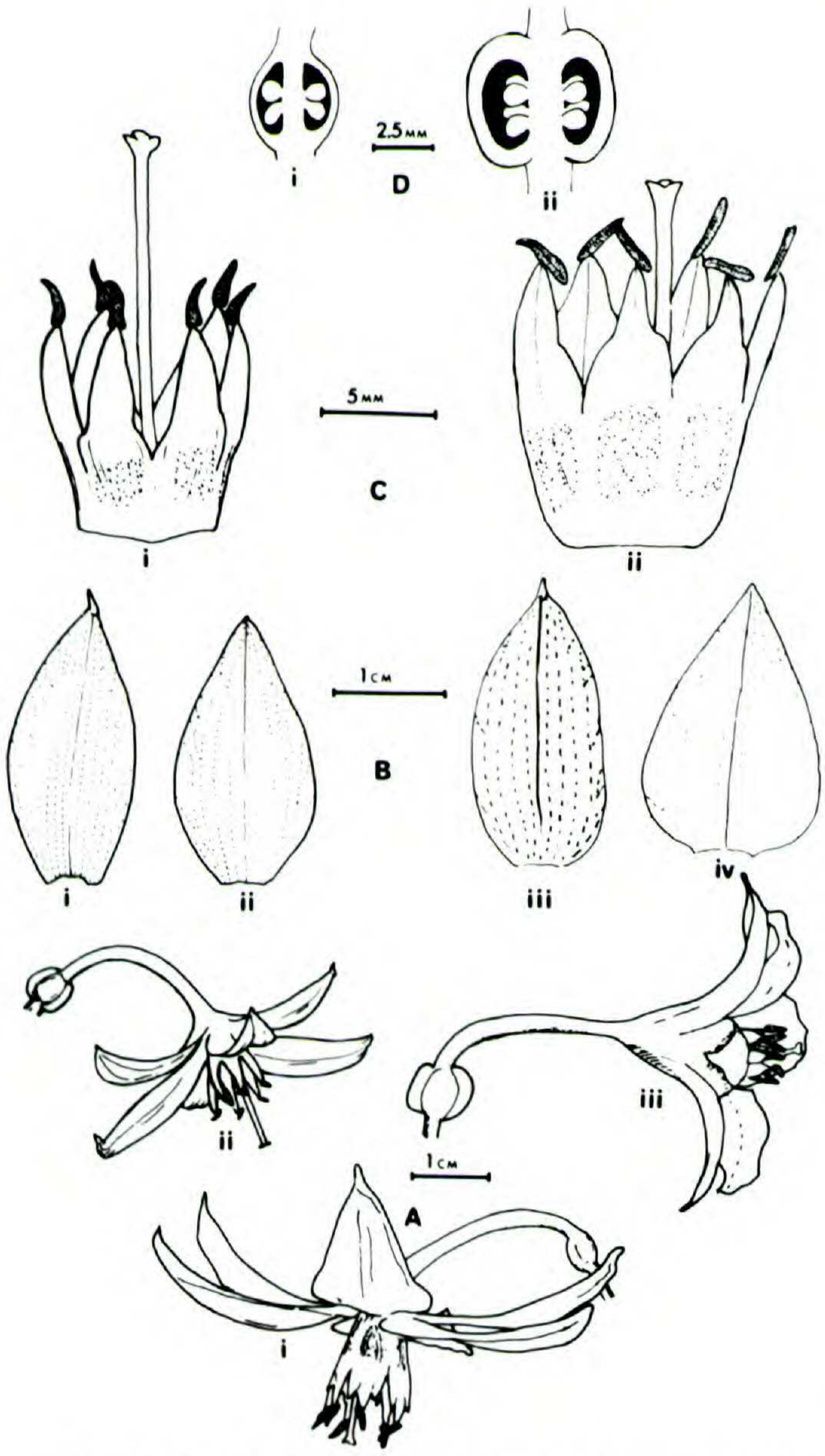


FIGURE 1. *Eucharis bouchei.*—A. Flowers.—i. Variety *dressleri* (holotype, *Meerow 1107,* FLAS). ii, iii. Variety *bouchei.*—ii. *Meerow 1125,* FLAS.—iii. *Meerow 1157,* FLAS.—B. Tepals, variety *bouchei.* i, ii. *Meerow 1125.*—i. Outer tepal.—ii. Inner tepal. iii, iv. *Meerow 1157.*—iii. Outer tepal.—iv. Inner tepal.—C. Staminal cups, variety *bouchei.*—i. *Meerow 1125.*—ii. *Meerow 1157.*—D. Ovaries, variety *bouchei,* longitudinal section.—i. *Meerow 1125.*—ii. *Meerow 1157.*

darienensis, found in Panamá and Darién provinces, is obtusely bidentate or lobed (Fig. 3D). The free filament constricts distally into a narrow (<2 mm wide) subulate portion. These two varieties occur in close proximity in one location near Cerro Campana in Panamá Province. The rare var. *dresslerii* (Fig. 1Ai), with its acutely toothed staminal cup (Fig. 3C) and non-trigonous ovary, occurs close to populations of var. *bouchei* near El Valle.

Northwesternmost populations in Panama representing var. *bouchei* have the most derived androecial morphology (Fig. 3A, B) relative to more southeastern populations (var. *darienensis,* Fig. 3D). The latter have staminal cups similar to the generalized morphology characteristic of Andean and Amazonian species of subg. *Eucharis.* This may indicate that general movement of *E. bouchei* in Central America has been away from the Colombian border.

The only other naturally occurring tetraploid *Eucharis* species known is the rare Colombian *E. bonplandii* (Kunth) Traub. It is separated from *E. bouchei* by its slightly glaucous leaves (all other *Eucharis* species have nonglaucous foliage), shorter petioles, and longer pedicels. *Eucharis bonplandii* is the northernmost species of *Eucharis* subg. *Eucharis* in South America.

Results of phenetic, karyotype, and preliminary electrophoretic analyses of *E. bouchei* and *E. bonplandii* are presented in this paper. These data offer insight into the evolutionary history of Central American *Eucharis* and the origins of tetraploidy in the genus, and provide a basis for understanding the enormous degree of phenotypic variation present within *E. bouchei.*

The use of electrophoretic analyses of isozyme variation in plant systematics has been extensive in recent years. The subject has been reviewed by Gottlieb (1971, 1977, 1981a, 1981b, 1982, 1984) and Crawford (1983, 1985). Unlike many morphological characters, which may be influenced by a great deal of environmental or developmental plasticity, the electrophoretic phenotype is more directly equitable with genotype (Crawford, 1983; Gottlieb, 1977).

Electrophoretic studies of tropical plants are few (Hamrick & Loveless, 1986; Heywood & Fleming, 1986; Sytsma & Schaal, 1985). Neither have plants of limited or rare distribution been widely investigated (Babbel & Selander, 1974).

Members of *Eucharis* are tropical monocots exclusive to rainforest understory. They are rare and widely dispersed in the wild. Studies of isozyme variation of any plant group fitting any one of these characteristics are very limited. Isozyme analyses of polyploid taxa are also not abundant (Crawford, 1985; Soltis & Rieseberg, 1986). Thus, an attempt to explore isozyme variation in natural polyploid *Eucharis* seemed a worthy avenue of investigation.

MATERIALS AND METHODS

PHENETIC ANALYSES

Principal component and hierarchical cluster analyses of 20 herbarium specimens of *Eucharis*

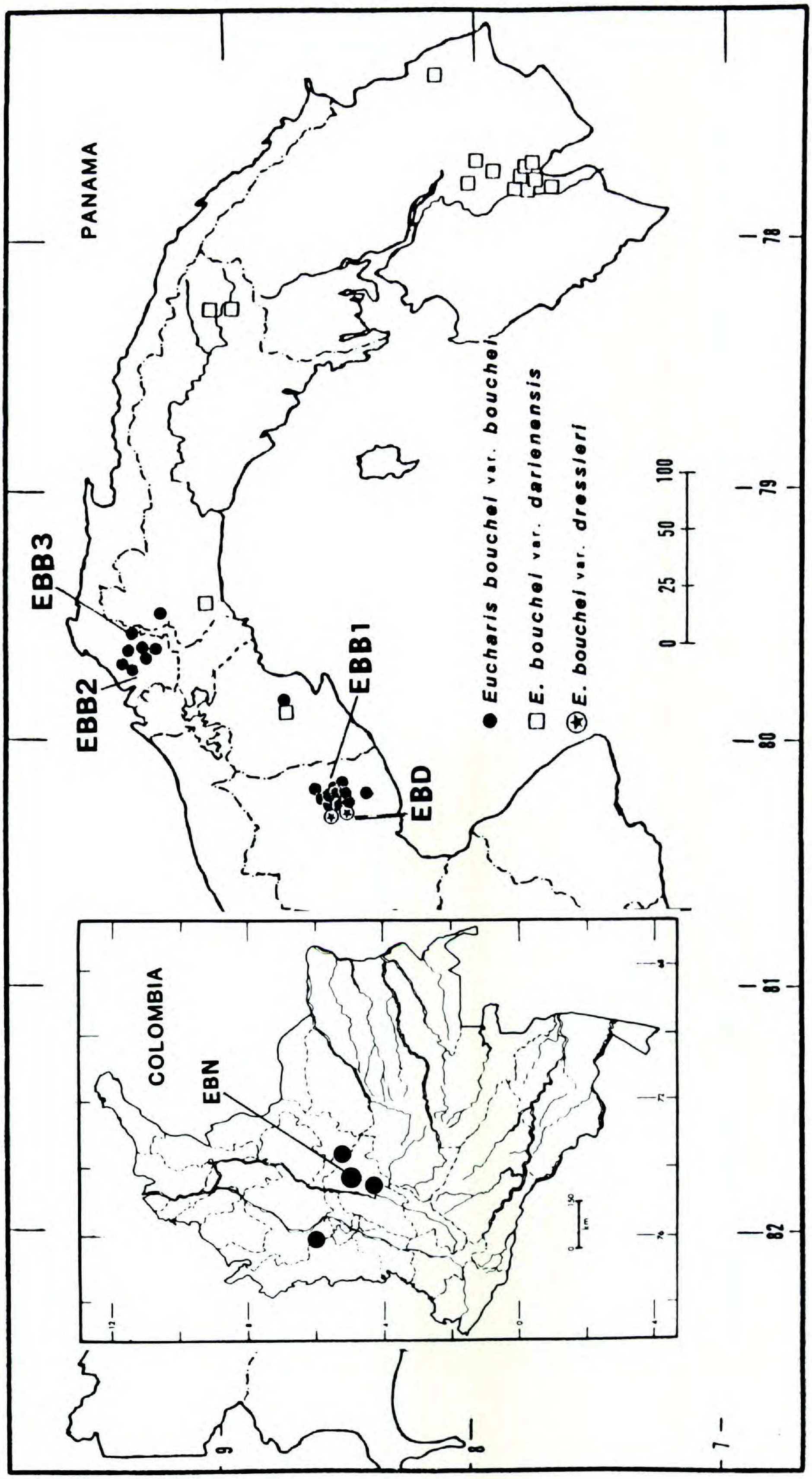


FIGURE 2. Distribution of *Eucharis bouchei* in Panama and *E. bonplandii* (Colombia, inset). Letter designations refer to populations analyzed electrophoretically (see Table 4).

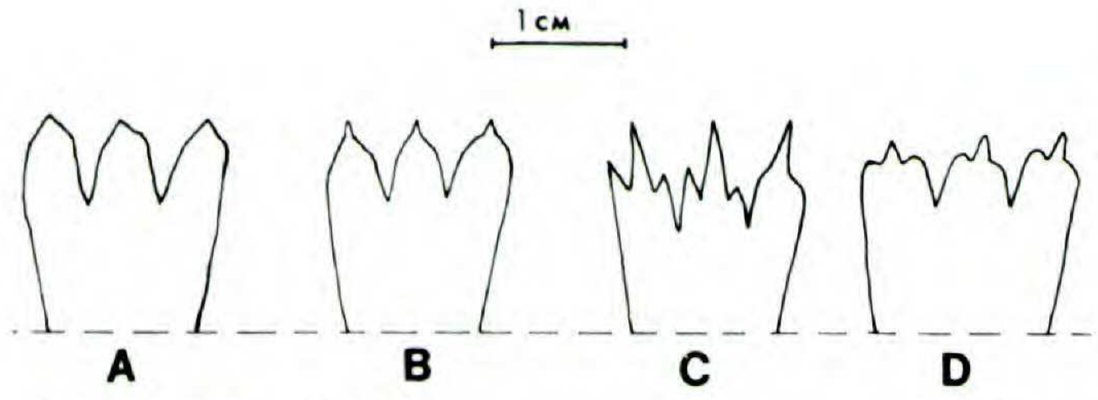


FIGURE 3. Staminal cup variation in *Eucharis bouchei*. A, B. Variety *bouchei*.—A. *Lewis et al. 2617* (MO).—B. *Allen 120* (US).—C. Variety *dressleri* (*Meerow 1107*, FLAS).—D. Variety *darienensis* (*Gentry & Mori 13945*, MO).

bouchei (Table 1) were conducted with CLUSTAN 2 vers. 2.1 (University of St. Andrews, Scotland) on the North Florida Regional Data Center (NERDC) system of the University of Florida. Three-dimensional scattergrams were constructed from PCA factor scores utilizing PCAPLOT, a program written by Bart Schutzman at the University of Florida. The small number of OTUs (operational taxonomic units) underscores the relative rarity with which *Eucharis* is encountered in the field. These 20 specimens represent the only specimens from which the full character set could be recorded. A number of additional specimens examined were collected in fruit and therefore were useless for these analyses.

Twenty-seven characters were used initially. The results suggested that some of these characters (e.g., all foliage characters, scape height, ovary length) were unreliable due to environmental plasticity, developmental variation, or specimen preparation. Although living material provides additional characters of potential utility (e.g., leaf surface texture, pigmentation pattern of the staminal cup), the inability to consistently determine these characters in dried specimens precluded their inclusion. Where any two characters were highly correlated (more than 80% correlation), which can result in data redundancy (Sneath & Sokal, 1973), one of the two was removed from the data matrix. In the final analyses, 17 floral characters (Tables 2, 3) were selected as the basic data set, of which 14 were continuous, quantitative characters. The remaining three qualitative characters were treated by assigning a numerical value for each character state. Since CLUSTAN would treat these values as continuous, the character states were numbered in a progressive transformation series, such that any two successive numbers would reflect putative character state relationship. These transformation series were constructed by study of morphological patterns and trends in the genus *Eucharis* and by comparative study with closely related genera of Amaryllidaceae.

Raw data were standardized using the "z-score" method (Sneath & Sokal, 1973) by which initial values for each character were replaced by stan-

TABLE 1. Operational taxonomic units (OTUs) for multivariate analysis of the *Eucharis bouchei* complex. CR = Costa Rica, G = Guatemala, P = Panama.

No.	Collection and Herbarium	Variety	Origin
1	*Alston 8727* (BM)	*dressleri*	P, Coclé
2	*Meerow 1107* (FLAS)	*dressleri*	P, Coclé
3	*Wendland 207* (GOET)	*darienensis*	G
4	*Sullivan 718* (MO)	*darienensis*	P, Darién
5	*Folsom 4402* (MO)	*darienensis*	P, Darién
6	*Folsom et al. 6582* (MO)	*bouchei*	P, Panamá
7	*Allen 5347* (US)	*bouchei*	CR
8	*Kirkbride & Hayden 305* (MO)	*bouchei*	P, Panamá
9	*Witherspoon & Witherspoon 8372* (MO)	*darienensis*	P, Panamá
10	*Duke & Elias 3661* (GH)	*darienensis*	P, Darién
11	*Gentry & Mori 13945* (MO)	*darienensis*	P, Darién
12	*Stern et al. 499* (GH)	*darienensis*	P, Darién
13	*Skutch 1585* (F)	*bouchei*	G
14	*Seibert 466* (MO)	*bouchei*	P, Coclé
15	*Lewis 2617* (MO)	*bouchei*	P, Coclé
16	*Witherspoon & Witherspoon 8736*	*bouchei*	P, Coclé
17	*Allen 1228* (GH)	*bouchei*	P, Coclé
18	*Mori & Kallunki 2014* (AAU)	*bouchei*	P, Colón
19	*Mori et al. 6586* (AAU)	*bouchei*	P, Colón
20	*Meerow 1158* (FLAS)	*bouchei*	P, Colón

dard deviations from the mean value for that character. A distance matrix was then calculated using squared euclidean distance (Cormack, 1971). In addition to PCA, cluster analysis using average linkage (unweighted pair group method (UPGMA) of Sneath & Sokal, 1973) was also applied to the 20 OTUs as a further test of phenetic relationship.

CHROMOSOME CYTOLOGY

Root tips were collected from living collections, pretreated for 2–3 hours at room temperature in 10 ppm solution of o-isopropyl-N-phenylcarbamate (Storey & Mann, 1967), rinsed in distilled water, fixed in 3 : 1 mixture of 95% EtOH and chloroform at 18°C for 24 hours, then stored after fixation in 70% EtOH at 18°C. Root tips were hydrolyzed in 1 N HCl at 50°C for 2–3 minutes, squashed, and stained with iron acetocarmine. Only temporary slides were made. Metaphase configurations were photographed on a Nikon Labophot photomicroscope with AFX-II camera attachment; haploid idiograms were constructed from photomicrographs.

As absolute chromosome length can vary appreciably from cell to cell due to differential effects of pretreatment (Tjio & Hagberg, 1951; Schlarbaum & Tsuchiya, 1984), relative length based on a value of 100 for the haploid complement was used to designate size class. Relative size classes are based on 80% or greater correlations between absolute size class (modified from Battaglia, 1955) and relative length (RL) of mitotic metaphase preparations of various species of *Eucharis, Eucrosia, Phaedranassa,* and other Amaryllidaceae with $2n = 46$, all of which have similar relative length ranges: large, RL $\geq$ 7.0 [absolute length (AL): > 10 μm]; moderately large, RL = 5.0–7.0 (AL: 7–10 μm); medium, RL = 3.5–5.0 (AL: 5–7 μm); small, RL $\leq$ 3.5 (AL: 2–5 μm). For tetraploid karyotypes, diploid RL values were halved to assign size class. Chromosome morphology, modified from Battaglia (1955), is defined as follows: metacentric, Arm Ratio (AR; long arm/short arm) = 1.00–1.10; near-metacentric, AR = 1.10–1.50; submetacentric, AR = 1.50–3.00; subtelocentric, AR = >3.00.

ELECTROPHORETIC ANALYSES

Population selection and sample size. Five populations were included in these analyses (Fig. 2; Table 4), representing all living collections of

TABLE 2. Characters used for multivariate analysis of *Eucharis* species.

1. Flower number
2. Limb spread (mm)
3. Length of free filament (mm)
4. Width of free filament (mm)
5. Width of stamen (mm)
6. Length of tube (mm)
7. Width of tube at throat (mm)
8. Length of outer tepal (mm)
9. Length of inner tepal (mm)
10. Width of outer tepal (mm)
11. Width of inner tepal (mm)
12. Staminal cup length (mm)
13. Staminal cup width (mm)
14. Toothing of staminal cup:
    1: Bidentate, teeth acute
    2: Bidentate, teeth obtuse
    3: Irregularly toothed
    4: Quadrate
    5: Lobed
    6: Edentate
15. Cleft of staminal cup:
    0: None
    1: < ⅕ length of cup
    2: ⅕–⅓ length of cup
    3: ⅓–½ length of cup
    4: > ½ length of cup
16. Relative length of teeth:
    0: Edentate
    1: < ½ length of filament
    2: ½ length of filament
    3: = length of filament
    4: > length of filament
17. No. ovules per locule

E. bouchei and *E. bonplandii* in cultivation at the University of Florida. These included three populations of *E. bouchei* var. *bouchei,* one from El Valle de Antón in Coclé Province of Panama, and one each from Cerro Brujo and Río Iguanita, respectively, in Colón Province; and one population of var. *dressleri,* also from El Valle. The fifth population represented *E. bonplandii,* a rare species from Colombia, also tetraploid. Sample size was one or three for each population (Table 4).

Unfortunately, the small sample size does not allow statistically significant exploration of genetic variation among these populations. Most electrophoretic studies in plant systematics have involved taxa of characteristically large population size in nature (see Crawford, 1983; Gottlieb, 1981a). Populations of *Eucharis,* however, are characteristically small. Many species of *Eu-*

TABLE 3. Data matrix for PCA and cluster analyses of the *Eucharis bouchei* complex.

	Character																
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	05	43	3.5	1.8	2.5	33.0	06.5	26.2	25.8	05.5	08.0	09.4	09.5	4	2	1	3
2	06	53	4.2	1.8	5.0	41.0	10.5	32.0	28.0	10.0	13.5	16.0	11.5	3	2	2	3
3	06	42	3.5	1.8	3.7	36.0	06.5	25.0	23.0	09.5	12.7	11.0	12.0	3	2	1	2
4	04	46	3.1	2.0	4.6	25.5	09.5	20.8	20.0	08.0	11.1	06.0	13.2	6	2	1	2
5	05	43	2.5	2.2	4.3	36.3	07.1	21.8	21.0	10.5	15.5	07.0	14.5	6	3	0	2
6	06	55	3.6	2.8	5.2	46.0	12.2	31.4	30.0	13.8	15.2	10.6	17.4	6	3	0	3
7	06	69	3.5	2.8	5.2	44.0	12.0	35.0	31.5	15.5	17.5	11.0	18.2	6	3	0	2
8	04	45	2.8	2.5	4.9	31.0	10.5	27.7	25.5	11.4	14.5	10.0	16.0	5	4	0	2
9	04	38	3.0	2.0	4.3	33.0	10.0	20.0	19.0	09.5	12.5	09.2	14.0	3	3	1	2
10	05	42	2.1	1.7	4.2	27.1	06.0	23.8	22.0	10.0	12.0	09.0	12.8	2	2	2	2
11	06	55	2.0	2.0	4.5	43.4	12.0	27.0	25.0	10.2	16.5	14.0	18.2	3	2	2	4
12	05	38	1.8	1.8	5.0	30.0	09.8	22.0	21.0	11.5	15.0	09.0	12.3	2	3	2	2
13	04	41	5.0	5.1	5.1	35.0	10.5	24.2	21.1	09.8	14.5	12.0	15.4	4	3	0	2
14	05	45	5.3	4.3	5.3	36.0	08.7	28.0	26.0	12.0	14.0	11.0	14.0	4	3	0	2
15	07	50	6.9	5.0	6.9	43.7	10.8	26.8	25.0	14.0	14.6	16.7	16.0	4	3	0	2
16	05	49	4.5	3.5	4.5	43.0	10.6	21.0	20.0	09.0	11.0	08.0	12.5	4	3	0	2
17	03	50	6.5	4.7	6.5	45.0	12.0	23.5	22.0	11.5	15.5	12.8	14.5	4	3	0	2
18	05	34	5.9	3.7	3.7	34.0	08.3	18.0	16.0	09.0	11.5	11.6	11.5	4	3	0	2
19	05	35	3.7	4.0	4.0	33.0	07.0	21.6	19.5	08.0	10.0	10.0	12.4	4	2	0	2
20	05	40	5.3	3.8	3.8	40.0	08.0	26.0	24.0	11.2	13.7	11.8	09.7	4	3	0	2

charis frequently occur as single, widely dispersed clumps (Meerow, 1986). Herbarium specimens of *Eucharis* regularly include some notation indicating the rarity of the plants encountered. Yet, if most *Eucharis* are primarily visited by trap-lining insects (sensu Janzen, 1971) flying long distances, as may be the case (Meerow, 1986), population size from the perspective of potential gene exchange may in fact be greater than otherwise expected from known population densities. Few workers have addressed the problem of how to apply electrophoretic data to rare plants of characteristically small population size. Nei (1978) presented modified formulas for unbiased genetic identities and distances that could be used for sample sizes as low as one. Nei stressed, however, that with a limited population sample, a large number of loci must be analyzed. The number of enzyme systems assayed in the present study is not sufficient for this purpose. Instead, individuals were scored for the presence or absence of putatively identical bands (Table 5). These scores were used to generate distance coefficients by the unweighted pair group method (Sneath & Sokal, 1973). Bands showing the same mobility, as determined by their position in the gel, were considered to be identical. The resulting dendrogram could then be compared with the results of phenetic analysis of morphological variation, as well as with the data derived from

TABLE 4. *Eucharis bouchei* and *E. bonplandii* populations examined electrophoretically.

Taxon	Designation	N	Collection Information	Voucher[a]
Eucharis bouchei var. *dressleri*	EBD	3	Panama, Coclé, El Valle de Antón	*Meerow 1107*
E. bouchei var. *bouchei*	EBB1	3	Panama, Coclé, El Valle de Antón	*Meerow 1125*
E. bouchei var. *bouchei*	EBB2	1	Panama, Colón, Río Guanche, Cerro Brujo	*Meerow 1157*
E. bouchei var. *bouchei*	EBB3	1	Panama, Colón, Río Iguanita	*Meerow 1158*
E. bonplandii	EBN	1	Colombia, Cundinamarca, vicinity of Bogotá	*Bauml 686* (HUNT)

[a] All vouchers deposited at FLAS unless otherwise indicated.

TABLE 5. Presence-absence data matrix for electrophoretic analysis of tetraploid *Eucharis*. Refer to Table 4 for population designations, Figure 14 for bands. (*) = used only for cladistic analysis. Blank space indicates data unavailable.

	Band																		
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	*18	*19
EBD	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	1
EBD	1	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1	1	1
EBD	1	0	0	0	1	1	0	0	0	0	0	0	1	1	0	0	1		
EBB1	0	0	1	1	1	1	1	0	0	0	0	0	1	1	1	1	0	0	1
EBB1	1	1	1	0	1	1	1	1	0	0	0	0	0	0	1	0	1		
EBB1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	1	0	1	1	1
EBB2	0	0	1	0	1	1	1	1	0	0	0	0	1	1	1	1	0	0	1
EBB3	1	0	0	0	1	1	1	1	0	0	0	0	0	0	1	0	1	0	1
EBN	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	0

comparative chromosome morphology. A similar method for analyzing isozyme data was used by Ashton et al. (1984) for *Shorea* (Dipterocarpaceae), and Chou et al. (1986) for several genera of bambusoid grasses. The data matrix was additionally subjected to cladistic analysis using PAUP by David Swofford (Illinois Natural History Survey). The "Wagner method" of simple parsimony (Farris, 1970; Kluge & Farris, 1969) was applied in constructing the cladogram, and *E. bonplandii* was designated as the outgroup for polarization of character states.

Of course, without the benefits of formal genetic analysis, there is no guarantee of genetic homology between any two bands of seemingly identical mobility. Future studies may allow more precise analysis of genetic variation within and among populations of tetraploid *Eucharis*.

Isozyme extraction and electrophoresis Crude extracts for isozyme electrophoresis were prepared by grinding ten 5 mm diameter leaf discs in 1 ml of extraction buffer [100 mM Tris-HCl, 10 mM DTT, 20% glycerol, and 1 mM PMSF adjusted to pH 6.8 (Hames & Rickwood, 1981)]. Extracts were centrifuged twice, for ten minutes and two minutes, and the supernatant was decanted by pipette after each centrifugation.

Electrophoresis was performed on a BIO-RAD Protean II polyacrylamide gel apparatus. Gel recipes were adopted from Hames & Rickwood (1981). Running gels were 0.75 mm thick and 7.5% acrylamide (10 ml 30% acrylamide-bis acrylamide, 10 ml 1.5 Tris-HCl at pH 8.8, 19.85 ml H_2O, 100 μl 10% ammonium persulfate, and 15 μl TEMED). A 2.5% acrylamide stacking gel (1 ml 30% acrylamide-bis acrylamide, 1.92 ml 0.5 M Tris-HCl at pH 6.8, 9 ml H_2O, 20 μl ammonium persulfate, and 7.5 μl TEMED) was employed. Running buffer was 25 mM Tris-glycine at pH 8.3 (Hames & Rickwood, 1981). A 20 μl sample of the supernatant was loaded into each stacking gel column. Gels were electrophoresed at a constant current of 50 mA until a blue indicator line (40 μl of bromophenol blue added to cathodal buffer) migrated off the anodal end of the gel, generally four to five hours.

Five enzyme systems were assayed: aspartate amino-transferase (AAT), glutathione reductase (GSSGR), malate dehydrogenase (MDH), phosphoglucoisomerase (PGI), and shikimate dehydrogenase (SKDH). Staining recipes of Vallejos (1983) were followed for AAT, PGI, and SKDH. The staining system for MDH was that of Shaw & Prasad (1970), and that of Kaplan (1968) was used for GSSGR.

Resolution of additional enzyme systems (galactose dehydrogenase, glutamate dehydrogenase, hexokinase, and isocitrate dehydrogenase) using the same buffer system were unsuccessful. Extracts of *Eucharis* leaf tissue are characteristically mucilaginous, which may impede electrophoretic separation or contribute to degradation of some enzymes after extraction. Also, cathodally migrating isozymes cannot be resolved in the same vertical acrylamide gel as anodally migrating isozymes.

RESULTS

PHENETIC ANALYSES

Principal Component Analysis (Figs. 4, 5; Table 6). Cumulative variance of 71.9% across 20 OTUs was resolved in the first three principal components (PCs). Characters 5 (stamen width),

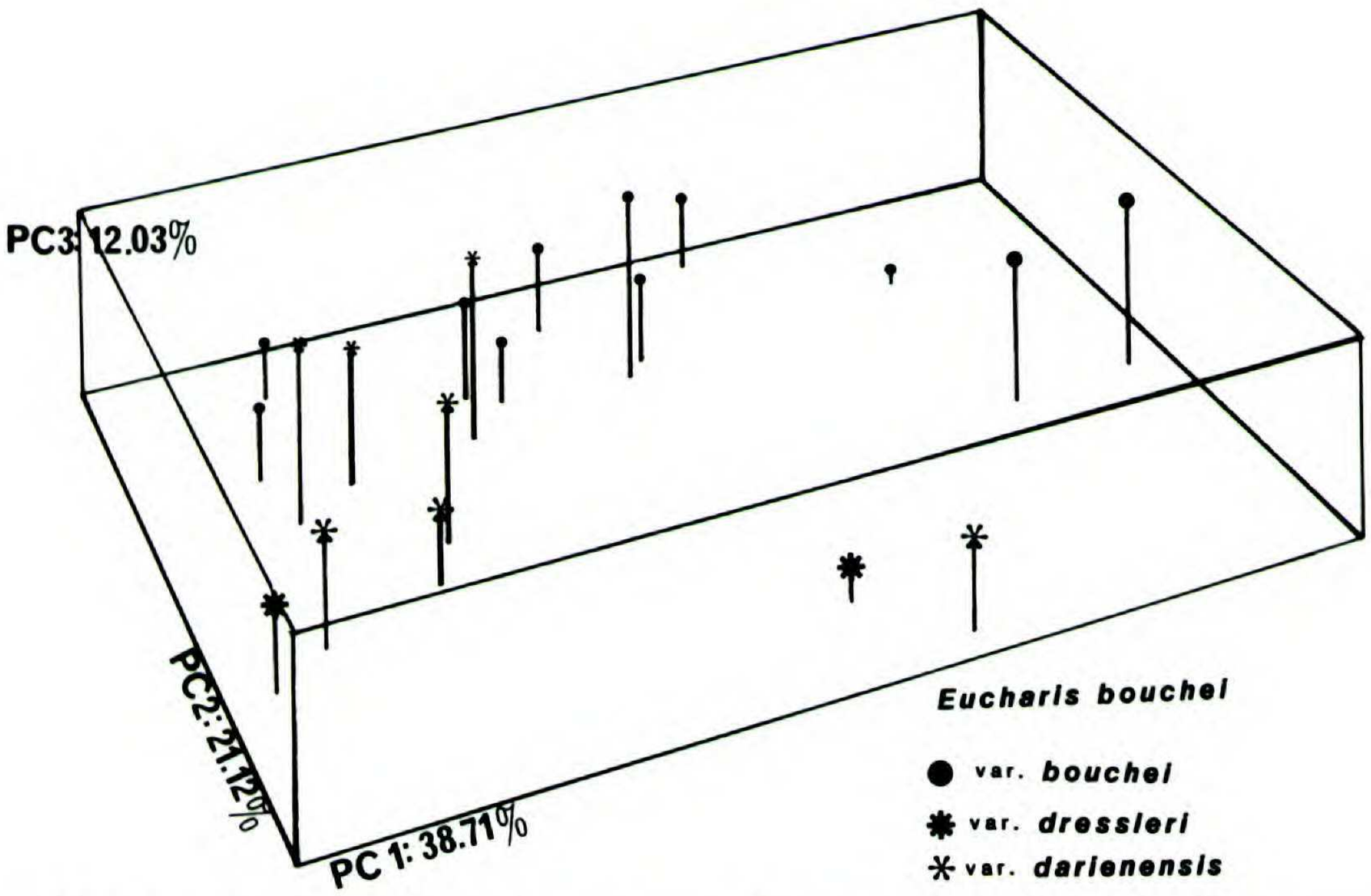


FIGURE 4. PCA scattergram based on variance across 17 floral characters in 20 OTUs representing *Eucharis bouchei.*

6 (tube length), 9 (inner tepal length), and 14 (toothing) contributed the greatest magnitude of variance to PC1, especially character 6. PC2 is largely a measure of outer tepal length (character 8), inner tepal width (11), staminal cup width (13), and toothing (14). Characters 1, 7, and 14 also substantially contributed to the variance reflected in PC2. Characters 2 (limb spread), 3 (length of free filament), 13 (staminal cup width), and 15 (toothing) were the most important sources of variance in PC3.

The three varieties of *E. bouchei* do not clearly resolve into three phenetic groups in Figure 4. Although var. *bouchei* shows a tendency to assemble along PC2 (21.1% total variance), this variety is still widely distributed along PC1 (38.7% total variance). One OTU each of var. *darienensis* (no. 11) and var. *dressleri* (no. 2) form an outlying group, as do OTUs 6, 7, and 15 of var. *bouchei.* Variety *darienensis* shows a measure of phenetic congruence, but intergrades with var. *bouchei.*

If the scattergram for the *E. bouchei* complex is rotated so that PC2 and PC3 are visually accentuated (Fig. 5), grouping of OTUs becomes largely a measure of androecial variance. In this scattergram, the three varieties are resolved more clearly, particularly var. *bouchei.* Variety *darienensis,* however, still intergrades with several OTUs of var. *bouchei,* but one of these OTUs (8) was collected from Cerro Campana in Panamá Province, an area of sympatry between these two varieties. The third (no. 7) is a Costa Rican collection.

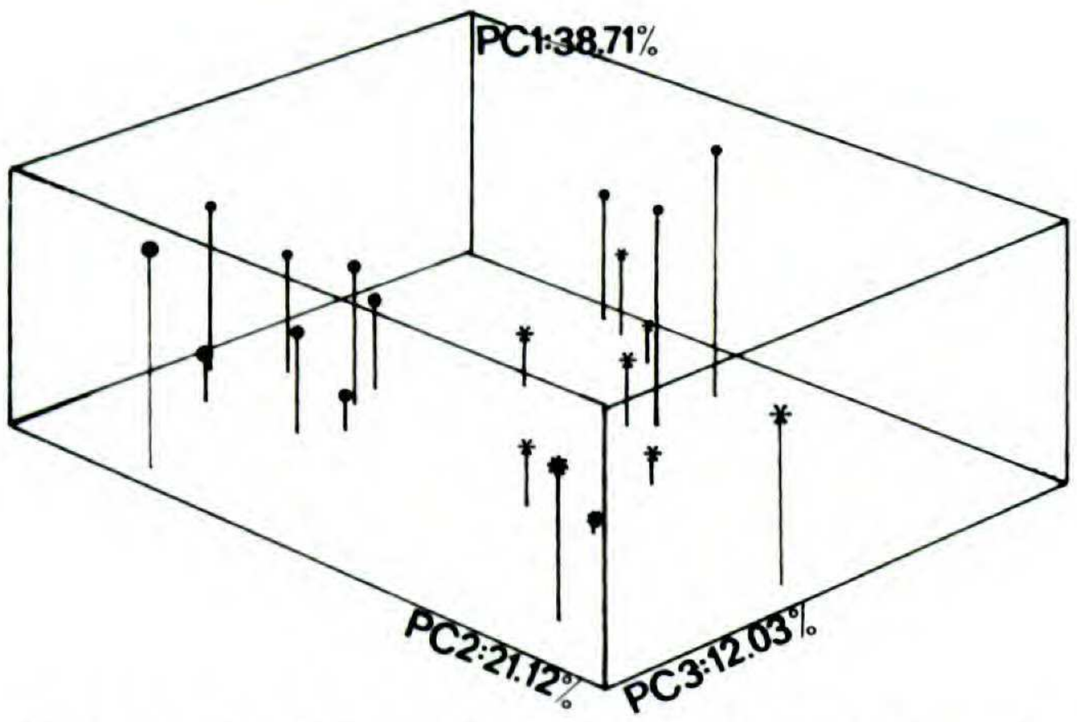


FIGURE 5. PCA scattergram based on variance across 17 floral characters in 20 OTUs representing *Eucharis bouchei,* with PC2&3 emphasized.

Cluster analysis (Fig. 6). Two major clusters are resolved in the UPMGA dendrogram, each fairly heterogeneous. The first clusters at a distance coefficient (DC) of 1.356. An outlying OTU (one of two representing var. *dressleri*) fuses with this cluster at DC 1.921. Within this first cluster, two subgroups emerge at DCs 1.207 and 1.213, respectively. The former is made up entirely of OTUs representing var. *darienensis.* The second represents var. *bouchei,* with the single exception of OTU 5 (var. *darienensis*). OTU 5 forms together with OTU 8 (var. *bouchei*) an outlying cluster to this second subgroup.

The second major cluster is formed at a DC of 2.502, near where all clusters finally merge (DC 2.779). This smaller cluster is more hetero-

TABLE 6. First three principal components for multivariate analysis of the *Eucharis bouchei* complex.

Character Number	Component Number		
	1	2	3
1	0.151	0.317	0.120
2	−0.265	−0.189	0.340
3	−0.244	0.112	−0.440
4	−0.197	−0.141	−0.147
5	−0.375	0.129	0.059
6	−0.615	0.099	0.133
7	0.032	−0.302	−0.116
8	−0.100	−0.336	−0.106
9	−0.404	0.069	−0.061
10	0.017	0.168	−0.239
11	−0.030	0.386	0.137
12	0.156	−0.062	−0.045
13	0.013	−0.353	−0.524
14	−0.232	−0.381	0.216
15	−0.184	0.322	−0.436
16	−0.007	0.056	−0.121
17	−0.046	−0.195	0.020
Percent of Variance	38.71	21.12	12.03

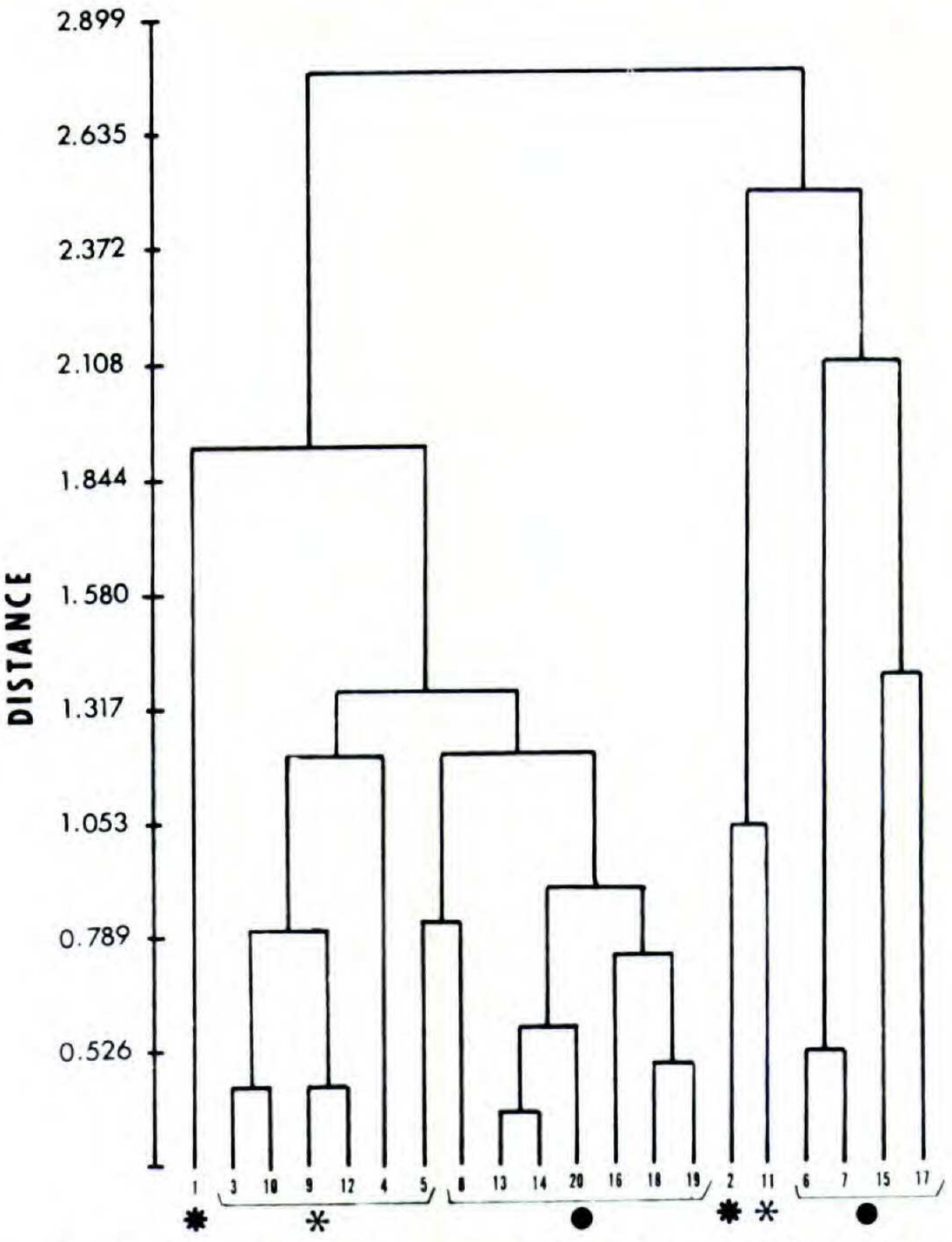


FIGURE 6. Cluster analysis dendrogram based on variance across 17 floral characters in 20 OTUs representing *Eucharis bouchei*. Refer to Table 1 for identification of OTUs.

geneous than the first, but four OTUs of var. *bouchei* cluster at a DC of 2.112. As in PCA, OTUs 2 and 11 (var. *dressleri* and *darienensis* respectively) form a phenetic group.

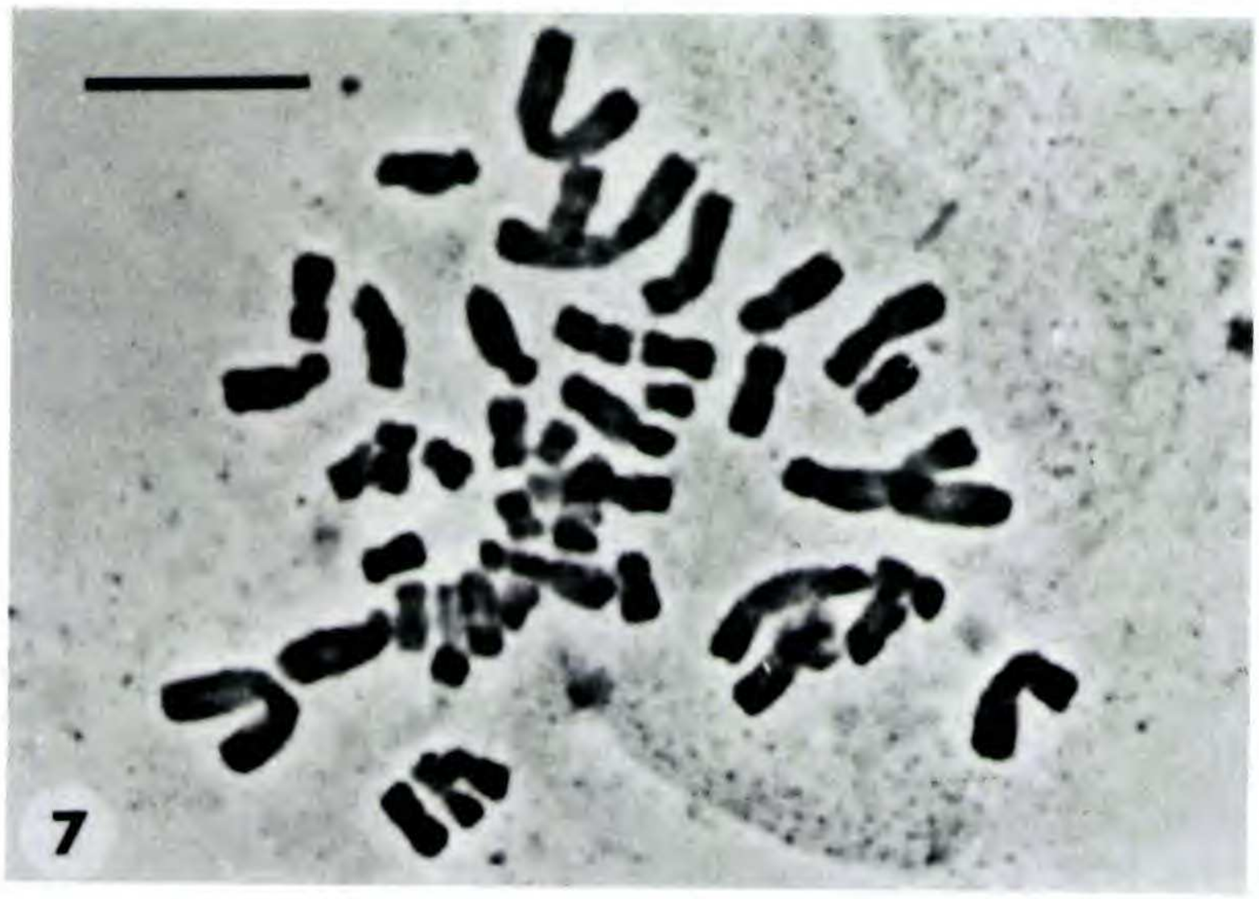
KARYOTYPE ANALYSIS

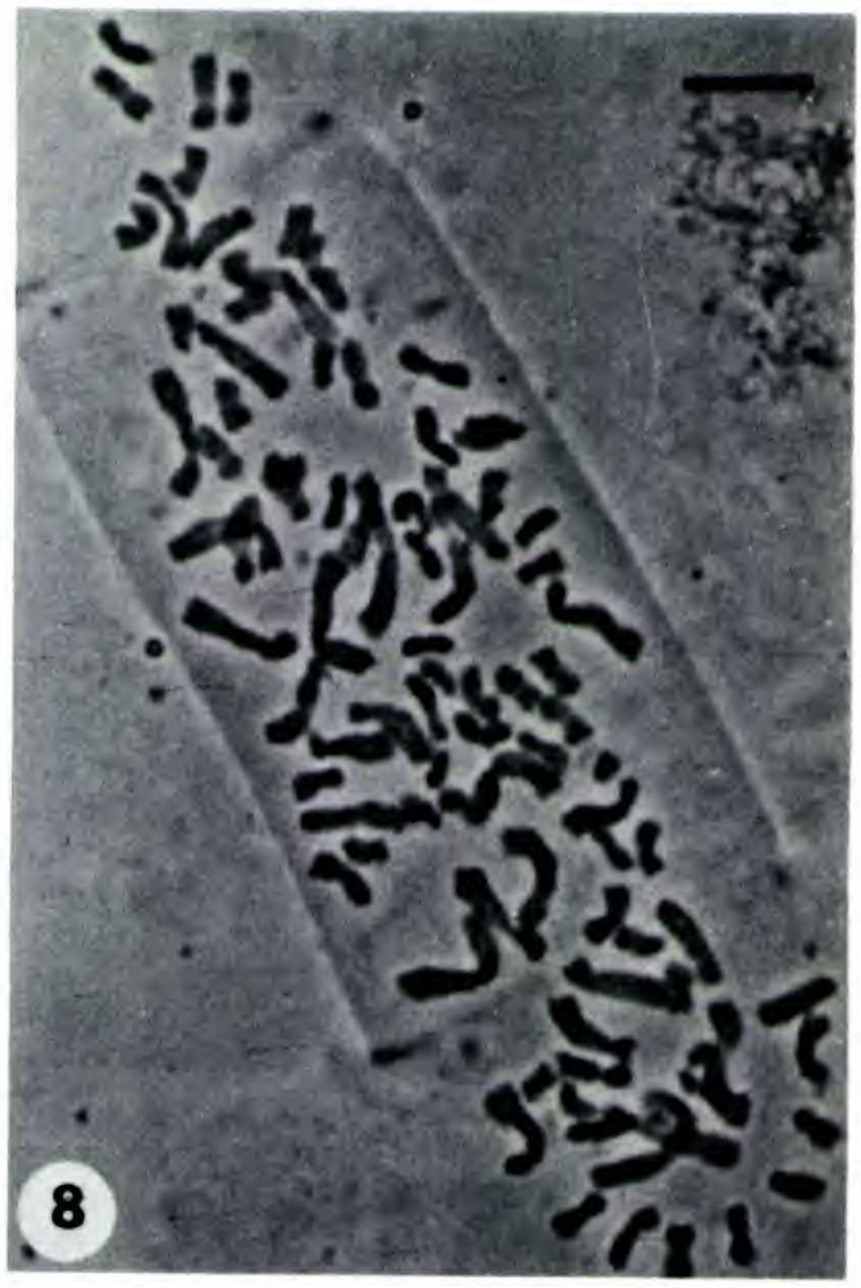
Tetraploidy in *Eucharis* is known so far to characterize only *E. bonplandii* and *E. bouchei*. Karyotypically, the tetraploid *Eucharis* species are strongly heteromorphic (Figs. 7–12; Table 7). Karyotypes of two geographically isolated and morphologically distinct populations of *E. bouchei* var. *bouchei*, from Coclé and Colón provinces of Panama (Figs. 9, 10, 12A, B; Table 7) are quite different. The second largest chromosome pair is submetacentric in the Cerro Brujo (Colón) population of *E. bouchei* (Figs. 10, 12B) and also in *E. bonplandii* (Figs. 11, 12C). *Eucharis bouchei* var. *dressleri* is an unstable tetraploid (Figs. 7, 8, 12D). Fifty percent of all root cells from which metaphase counts were obtained had 46 chromosomes.

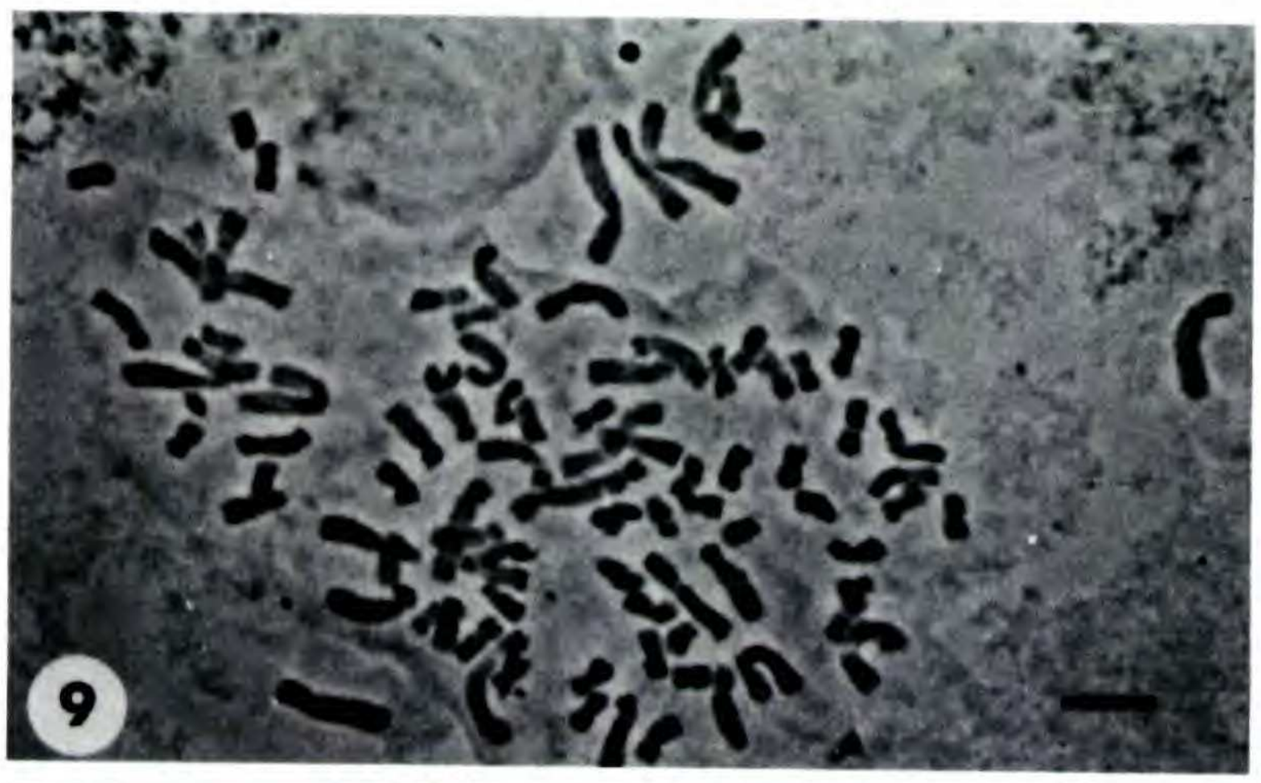
ELECTROPHORETIC ANALYSES

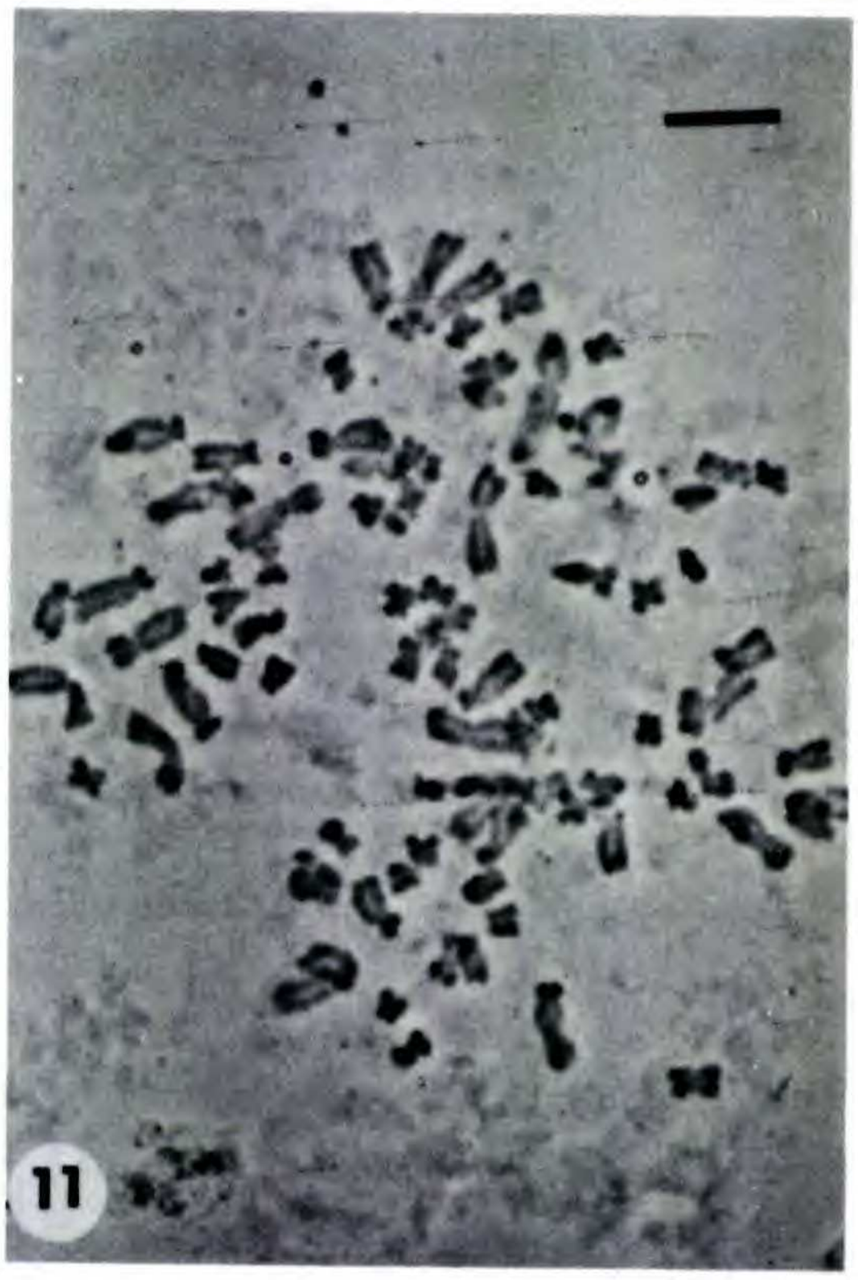
Of the five enzyme systems assayed, only SKDH was monomorphic across all populations of *E. bouchei* and *E. bonplandii*. Only polymorphic loci are discussed below and diagrammed in Figure 14.

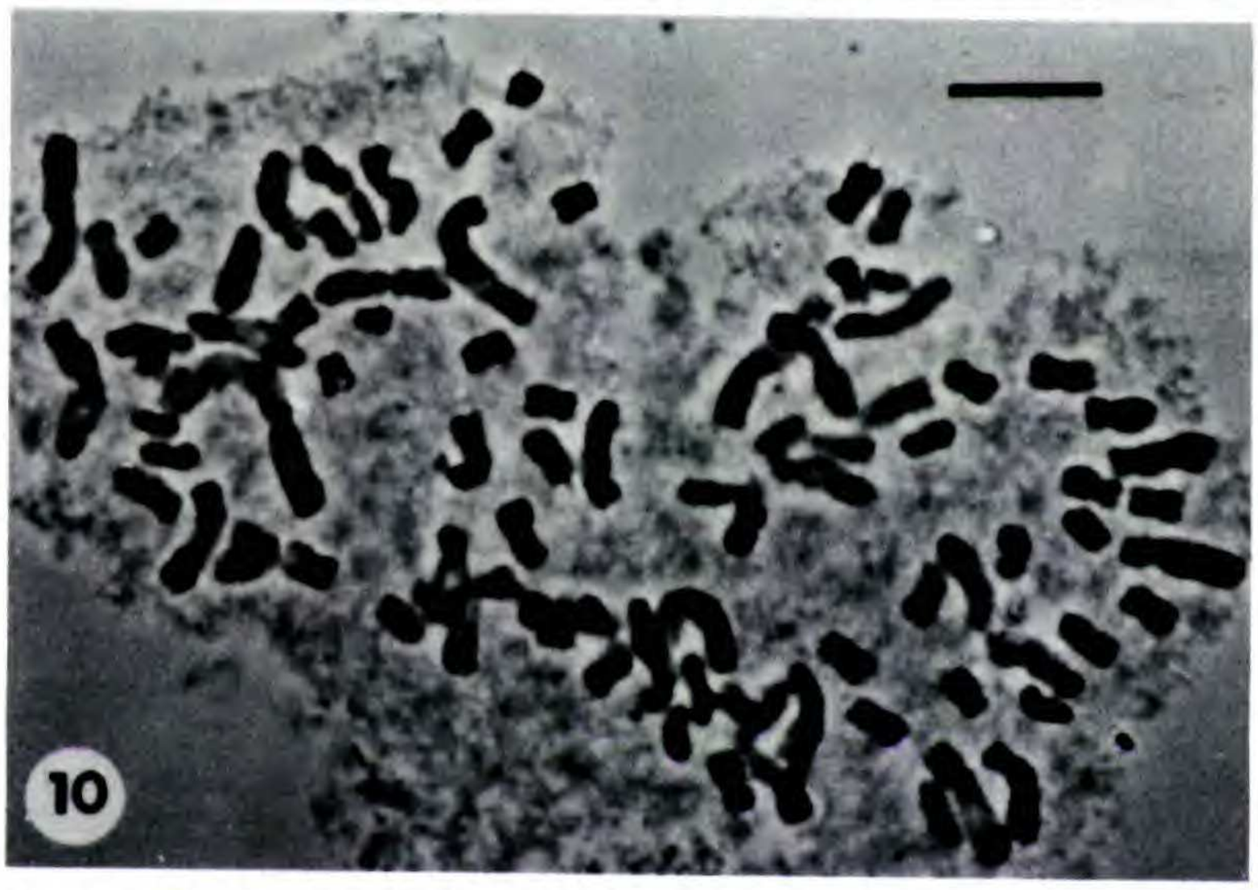
AAT (Figs. 13A, 14). Two well-separated regions of activity were resolved for AAT, one rapidly migrating anodally (AAT-1) and the other (AAT-2) considerably slower; these probably represent two different loci of this dimeric enzyme. Electromorphs at both loci were considerably more complex than in diploid species of *Eucharis* (Meerow, 1986). Two putative alleles are inferred from the phenotypes of AAT-1 in the *E. bouchei* complex. Each "allele" of AAT-1 in all *Eucharis* characteristically resolves as two very closely spaced bands. This may be the result of breakdown products forming after extraction (see Fig. 1 in Shields et al., 1983). Electromorphs of pollen of diploid ($2n = 46$) *Eucharis* (Meerow, unpubl.) also showed this banding pattern. Were each component band of the doublet a distinct allele, pollen would be expected to show only one of the two (Gottlieb, 1982, 1984). Alternatively, if the high diploid chromosome number ($2n = 46$) of *Eucharis* was originally tetraploid-derived (Meerow, 1987a), the doublet banding pattern may reflect duplication of the genome and would show up in pollen.











FIGURES 7–11. Root-tip cell mitotic metaphase configurations of tetraploid *Eucharis* species. 7, 8. *E. bouchei* var. *dressleri*.—7. Diploid cell.—8. Tetraploid cell.—9. *E. bouchei* var. *bouchei* from Colón Province in Panama.—10. *E. bouchei* var. *bouchei* from Coclé Province in Panama.—11. *E. bonplandii*. Two small chromosomes are outside the figure frame. All scales = 10 μm.

Band a was the most common "allele" of AAT-1, found in all individuals analyzed except for two putative homozygotes for "allele" c (the Cerro Brujo population and one individual of the El Valle population of var. *bouchei*). Variety *dressleri* and *E. bonplandii* are homozygous for "allele" a. The Río Iguanito individual of var. *bouchei* is homozygous for "allele" b, while two individuals of the El Valle population show a putatively heterozygous phenotype with apparent heterodimerization.

Four *E. bouchei* individuals resolved a four-banded electromorph at the putative AAT-2 locus. Bands f and g were found only in *E. bon-*

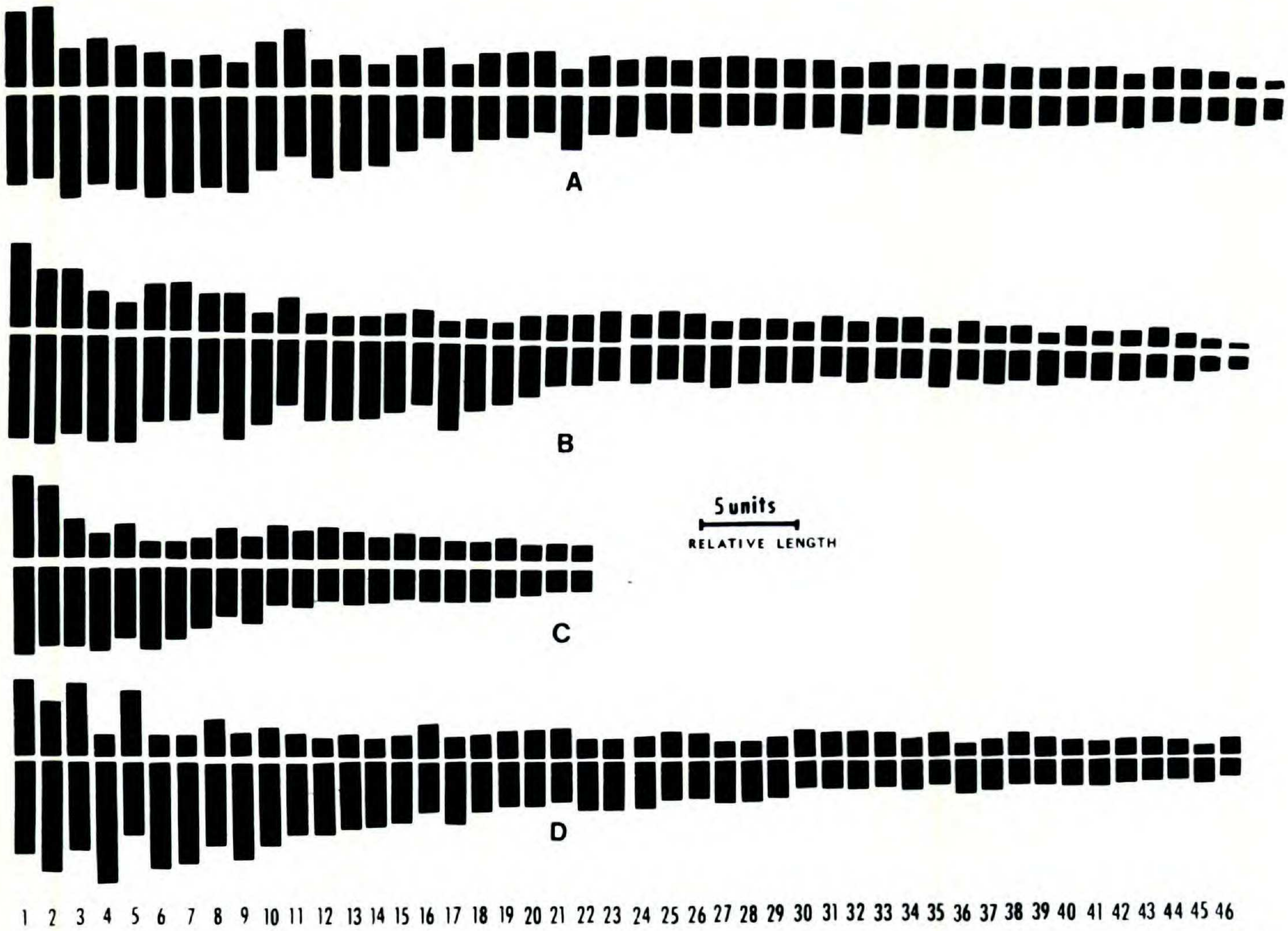


FIGURE 12. Haploid idiograms of tetraploid *Eucharis* karyotypes.—A. *E. bouchei* var. *bouchei* from Coclé Province in Panama.—B. *E. bouchei* var. *bouchei* from Colón Province in Panama.—C. *E. bouchei* var. *dressleri,* diploid cell.—D. *E. bonplandii.*

plandii. Only two bands were observed in var. *dressleri* (an unstable tetraploid), representing "alleles" a and b or b and c, and one individual of var. *bouchei* from El Valle ("alleles" c and d). All other individuals of *E. bouchei* resolved a four-banded electromorph for AAT-2. Band e was found in one of the three individuals of var. *bouchei* from El Valle. As all diploid species of *Eucharis* species resolve only a two-banded electromorph for this isozyme (Meerow, 1986), it was inferred that the proliferation of bands within *E. bouchei* represented the additive effects of tetraploidy (Crawford, 1983, 1985; Gottlieb, 1982).

MDH (Figs. 13B, 14). Malate dehydrogenase characteristically forms complex banding patterns that require genetic analysis to decipher (Kirkpatrick et al., 1985; Torres & Mau-Lastovicka, 1982). Consequently, no attempt is made to infer genotypes in any detail from the banding pattern. However, the phenotype of the most anodal bands in *E. bonplandii* suggests the presence of two alleles and their heterodimer [pollen of this species resolved only a single band at this locus in a repetitive run (Meerow, unpubl.), supporting this interpretation]. The intensity of the bands in the two most cathodal regions of several individuals suggests dosage effects in putative homozygotes (two individuals of EBB1, and EBB3 in Figs. 12B, 13).

GSSGR (Fig. 14). Two bands were observed in the single locus resolved for GSSGR, all individuals manifesting one or the other.

PGI (Fig. 14). Only a single region of activity was resolved for PGI. Two bands were observed, but the more anodal one was found only in the putative heterozygotes (two individuals of *E. bouchei* var. *dressleri,* and one of var. *bouchei* from El Valle), and in *E. bonplandii.*

Isozyme relationships. The nine individuals for which electrophoretic phenotypes were resolved were coded for presence or absence of the numbered bands in Figure 14, creating a data set of 19 characters. Phenotypes for PGI were not included in the cluster analysis, as data were not available for all nine individuals. The resulting

TABLE 7. Karyotype data, *Eucharis bouchei* and *E. bonplandii*. All vouchers deposited at FLAS unless otherwise stated.

Taxon, Voucher, & Fig. No.	Chromosome Number	Chromosome Size Range (μm)	Chromosome Size Range (relative length)[a]	Chromosome Size[b] Groups				Chromosome[c] Morphology				
				L	ML	M	S		L	ML	M	S
Eucharis bouchei var. *dressleri*[d] (*Meerow 1107*) Figs. 7, 8, 12C	46, 92	2.4–11.9	2.0–9.7	4	10	14	18	m:	4		2	
								nm:			6	10
								sm:		4	6	8
								st:		6		
E. bouchei var. *bouchei* (*Meerow 1157*) Figs. 9, 12B	92	1.6–15.5	0.5–5.3	14	16	14	48	m:				2
								nm:	2		2	18
								sm:	10	6	6	28
								st:	2	10	6	
E. bouchei var. *bouchei* (*Meerow 1125*) Figs. 10, 12A	92	2.0–10.4	0.8–4.5	10	18	16	48	m:	2	2	4	6
								nm:	2		6	28
								sm:	6	8	4	14
								st:		8	2	
E. bonplandii (*Bauml 686*, HUNT) Figs. 11, 12D	92	1.9–9.7	1.0–4.9	14	12	24	42	m:				6
								nm:	6		6	18
								sm:	2	4	16	18
								st:	6	8	2	

[a] Based on a value of 100 for the haploid complement.
[b] L = long, ML = moderately long, M = medium, S = small.
[c] m = metacentric, nm = near-metacentric, sm = submetacentric, st = subtelocentric.
[d] Diploid cell analyzed.

UPGMA dendrogram (Fig. 15) and cladogram (Fig. 16) illustrate the isozyme relationships among these individual plants. As might be expected, both trees are similar in topology. All three individuals of *E. bouchei* var. *dressleri* (EBD) cluster at a distance coefficient (DC) of only 0.118, indicating their close isozyme relationship. The El Valle (Coclé Province) population of var. *bouchei* (EBB1) is rather diverse in its patterns of isozyme variation. Two individuals are similar, clustering at a DC of 0.118. This cluster then fuses with the Río Iguanita (Colón Province) individual of var. *bouchei* (EBB3) at a DC of 0.176. The remaining individual of El Valle var. *bouchei* EBB1 shows greater isozyme relationship to Cerro Brujo (Colón Province) var. *bouchei* EBB2 than other convarietal individuals from El Valle. This heterogeneous cluster then fuses with var. *dressleri* (EBD) at a DC of 0.431, followed by the other El Valle individuals (EBB1) and the single Río Iquanita (EBB3) individual (DC 0.443). The single individual representing *E. bonplandii* (EBN) remains a distant outlyer from all plants of *E. bouchei*, joining the latter species at a DC of 0.625.

The cladogram based on isozyme data was 27 steps long with a consistency index (Kluge & Farris, 1969; CI = total length minus homoplasies, divided by total length) of 0.704. The cladogram supports a monophyletic origin of *E. bouchei* var. *dressleri* (EBD) from var. *bouchei* (EBB1) in Coclé Province, but suggests that var. *bouchei* may be polyphyletic. The Río Iguanita (Colón Province, EBB3) individual of var. *bouchei* forms a monophyletic group with two individuals of El Valle (Coclé province, EBB1) var. *bouchei*, while the other Colón individual (Cerro Brujo, EBB2) forms a monophyletic group with the third El Valle individual.

DISCUSSION

The Central American *E. bouchei* complex does not resolve into discrete phenetic groups. Staminal cup morphology, however, does separate varieties to a fair degree (Fig. 5). Floral size characters in this group (Figs. 4, 6) do not succeed as well in resolving phenetic groups.

Chromosome number is very stable in *Eucharis*, and polyploidy is infrequent. The origins of the polyploids (i.e., whether auto- or alloploid)

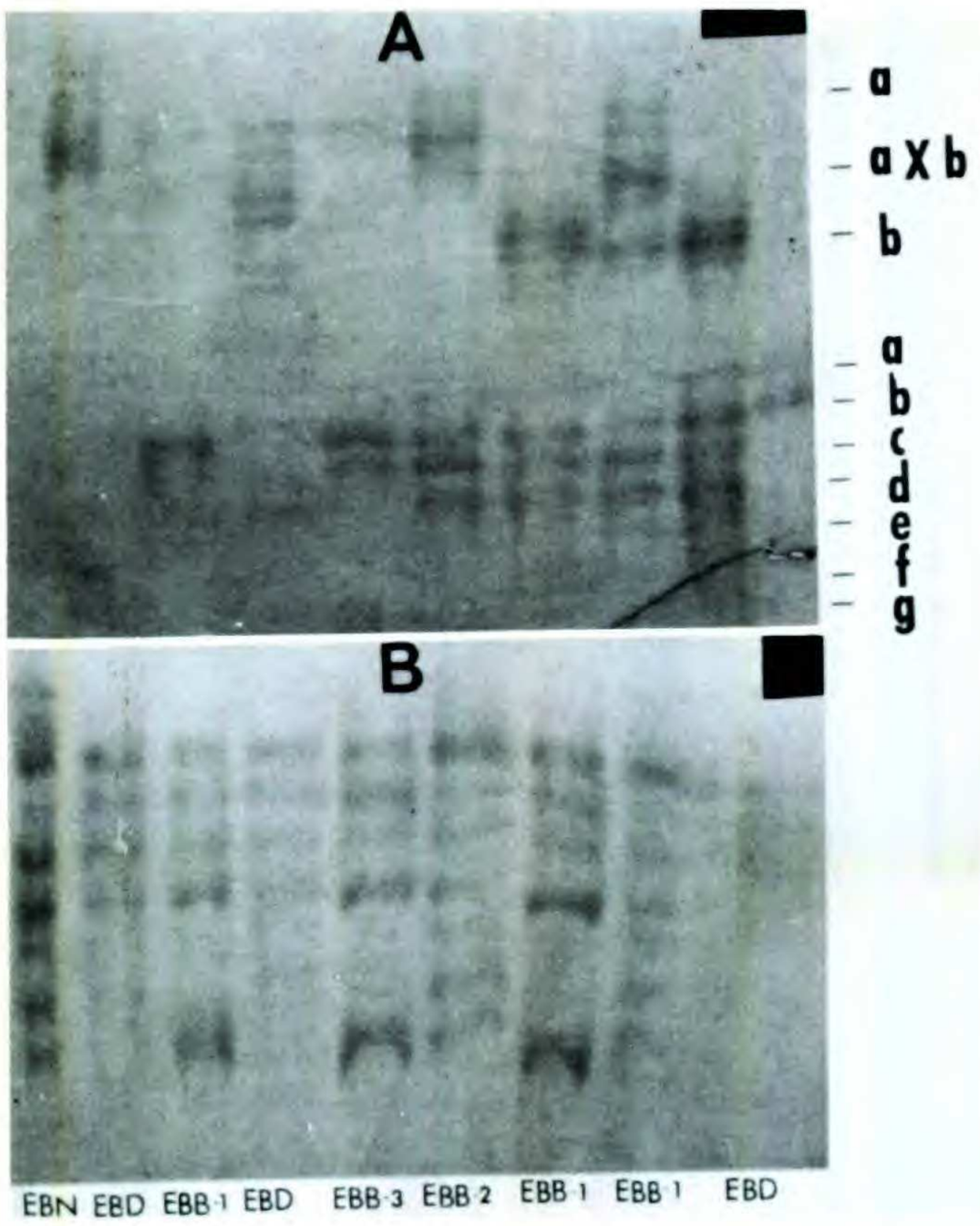


FIGURE 13. Representative gels for electrophoretic analysis of *Eucharis bouchei* complex.—A. Aspartate amino transferase (AAT).—B. Malate dehydrogenase (MDH). Anodal end of gel is at top of figure. Where no activity is apparent, it was subsequently resolved in repetitive runs. Lower case letters to right refer to putative alleles discussed in text. Refer to Table 4 for population designations.

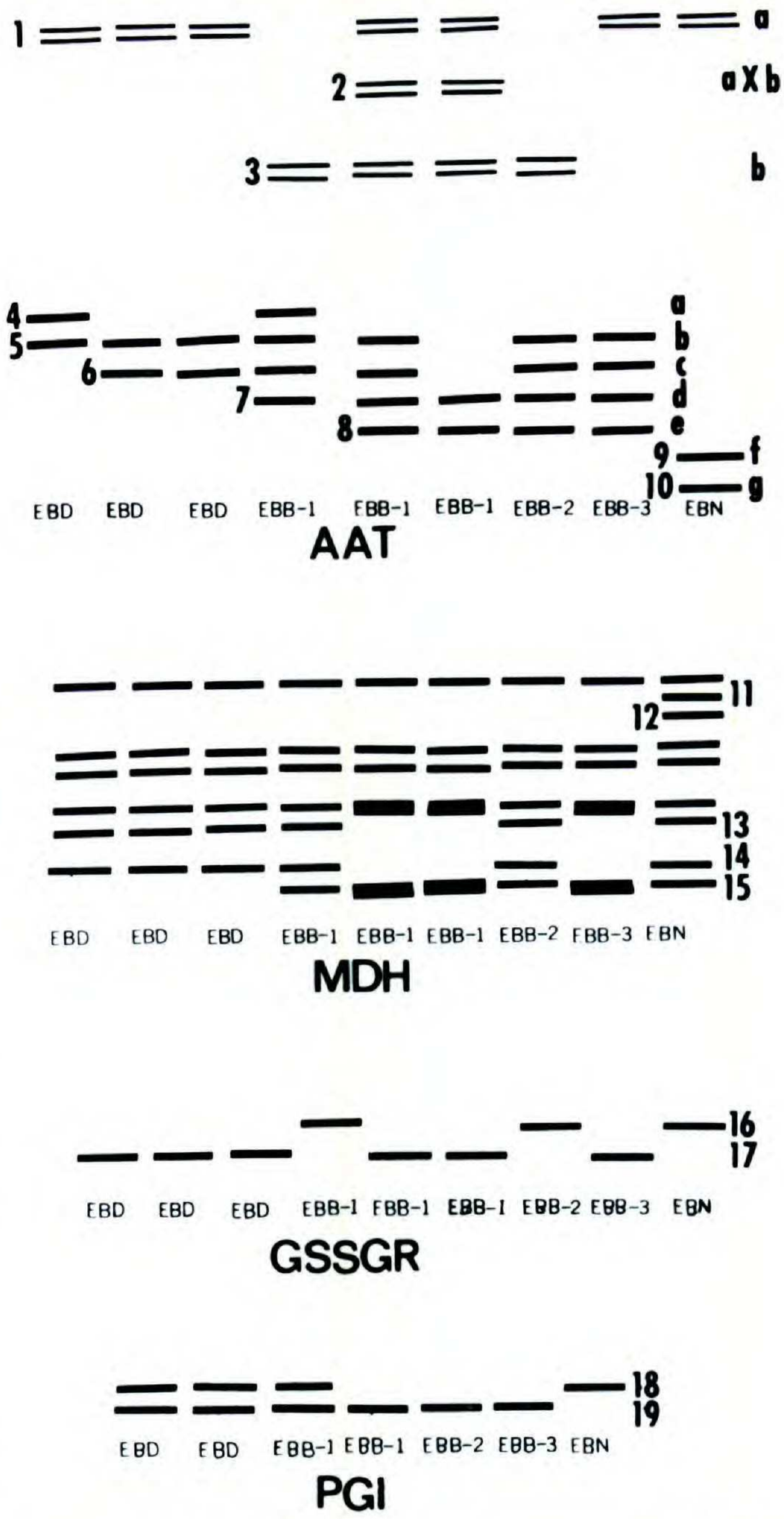


FIGURE 14. Electrophoretic phenotypes at all polymorphic loci in the *Eucharis bouchei* complex. Anodal end at top. Lower case letters to right refer to putative alleles discussed in text. Numbers refer to bands coded in presence/absence data matrix. Refer to Table 4 for population designations. AAT = aspartate amino transferase, MDH = malate dehydrogenase, GSSGR = glutathione reductase, PGI = phosphoglucoisomerase.

are inconclusive (attempts to secure meiotic figures have been unsuccessful), but the high levels of morphological diversity in *E. bouchei* might suggest that they are alloploids. Differences in chromosome morphology among the populations of *E. bouchei* examined (Table 7) suggest that structural changes in the chromosomes may have been important in interpopulational divergence. *Eucharis bouchei* var. *dressleri* is an unstable tetraploid. Somatic cells of the root tips have both tetraploid (92) and diploid (46) counts. Snoad (1955) reported karyotype instability in *Hymenocallis narcissiflora* (Jacq.) Macbr. (Amaryllidaceae), but aneuploid numbers as well as polyploid counts were observed in the cells of the latter species.

Polyploid species of *Eucharis* do not show any marked effects of increased chromosome number beyond an increase in size of root cells and stomata, and slight thickening of the leaf blades. *Eucharis bonplandii,* in addition, develops a glaucous bloom on the leaves in strong light, a novel characteristic for the genus.

Eucharis bouchei var. *bouchei,* as presently conceived (Meerow, 1986), is diverse in floral morphology, chromosome morphology, and patterns of isozyme polymorphisms. Cladistic relationships based on the isozyme data indicate that this polymorphic variety may even be polyphyletic. Morphologically EBB3 is similar to "typical" El Valle var. *bouchei* (Fig. 1Aii). Though floral morphological differences exist between the El Valle and Cerro Brujo populations of var. *bouchei* (Fig. 1Aiii), they are not discontinuous

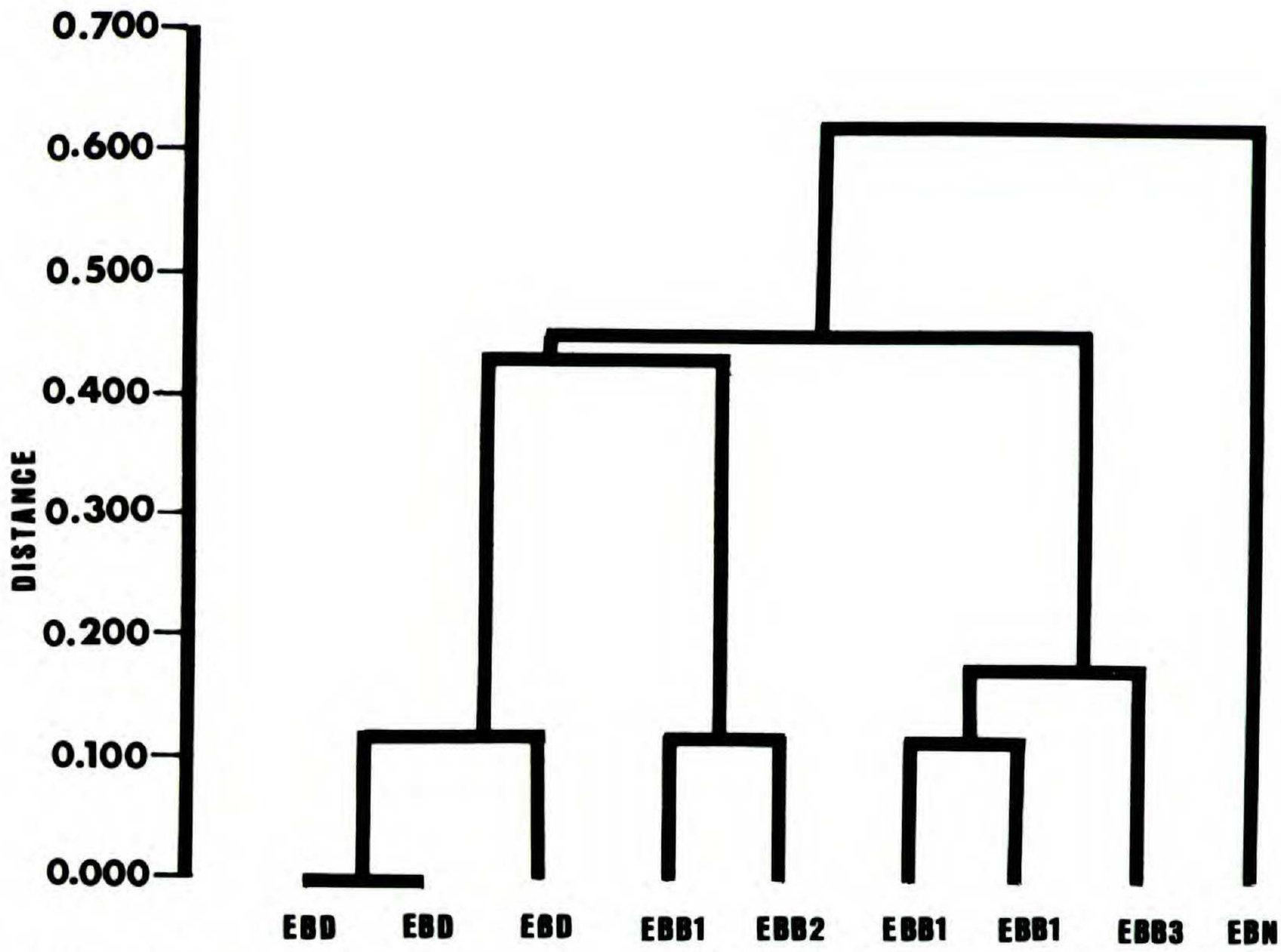


FIGURE 15. UPGMA dendrogram of tetraploid *Eucharis* isozyme phenotypes. Refer to Table 4 for population designations.

enough to warrant a clear differentiation of a fourth variety in the species. The two individuals representing different populations from Colón Province each have closer isozyme relationship to Coclé var. *bouchei* than they have to each other, while the Coclé population itself appears isozymically diverse. Cladistic relationships based on this data imply that Coclé populations of var. *bouchei* may be of heterogeneous ancestry. The cladogram (Fig. 16) also points to Colón Province as the likely origin of those ancestors. Divergence between and among the Coclé populations and those in Colón Province, presumably mediated by geographic isolation, may thus be an ongoing process.

On the basis of staminal cup morphology, I hypothesize that *Eucharis bouchei* has been steadily migrating away from the Colombian border (Meerow, 1986). The Cerro Brujo population of *E. bouchei* var. *bouchei* may represent an intermediate point in the divergence of a new geographical race of *E. bouchei.* The best test of this hypothesis would be the results of isozyme analysis of *E. bouchei* var. *darienensis,* the one variety for which material is not presently available. Variety *darienensis* occurs closer to the Colombian border than any other population of *E. bouchei* and has the most generalized staminal cup morphology relative to *Eucharis* as a whole. If my hypothesis is correct, var. *darienensis* should have the lowest genetic identity with populations of either var. *bouchei* or var. *dressleri* from Coclé Province, and higher identity with populations of var. *bouchei* from Colón Province.

Colón populations of *E. bouchei* are geographically intermediate between most populations of var. *darienensis* and the Coclé populations of var. *bouchei* (Meerow, 1986). The two varieties come into close proximity in the Cerro Campana area in Panamá Province. Colón populations may therefore also be genetically intermediate between the two varieties. Segregating genotypes in such a case could produce populations exhibiting a mosaic of varying genetic identity, some close to Coclé var. *bouchei,* others perhaps closer to var. *darienensis.* Further testing of this hypothesis with var. *darienensis* and larger numbers of populations and individuals is necessary.

Eucharis bouchei var. *dressleri* occurs sympatrically as a rare morph with populations of var. *bouchei.* The origin of var. *dressleri* may be the first step in sympatric speciation. This variety shows greatest isozyme relationship with certain individuals of the Coclé population of var. *bouchei.* In this regard, the presence of a second band for PGI in certain individuals of var. *dressleri* and El Valle var. *bouchei* may be significant (Fig. 14), but a larger number of individuals must be assayed to confirm this observation. This va-

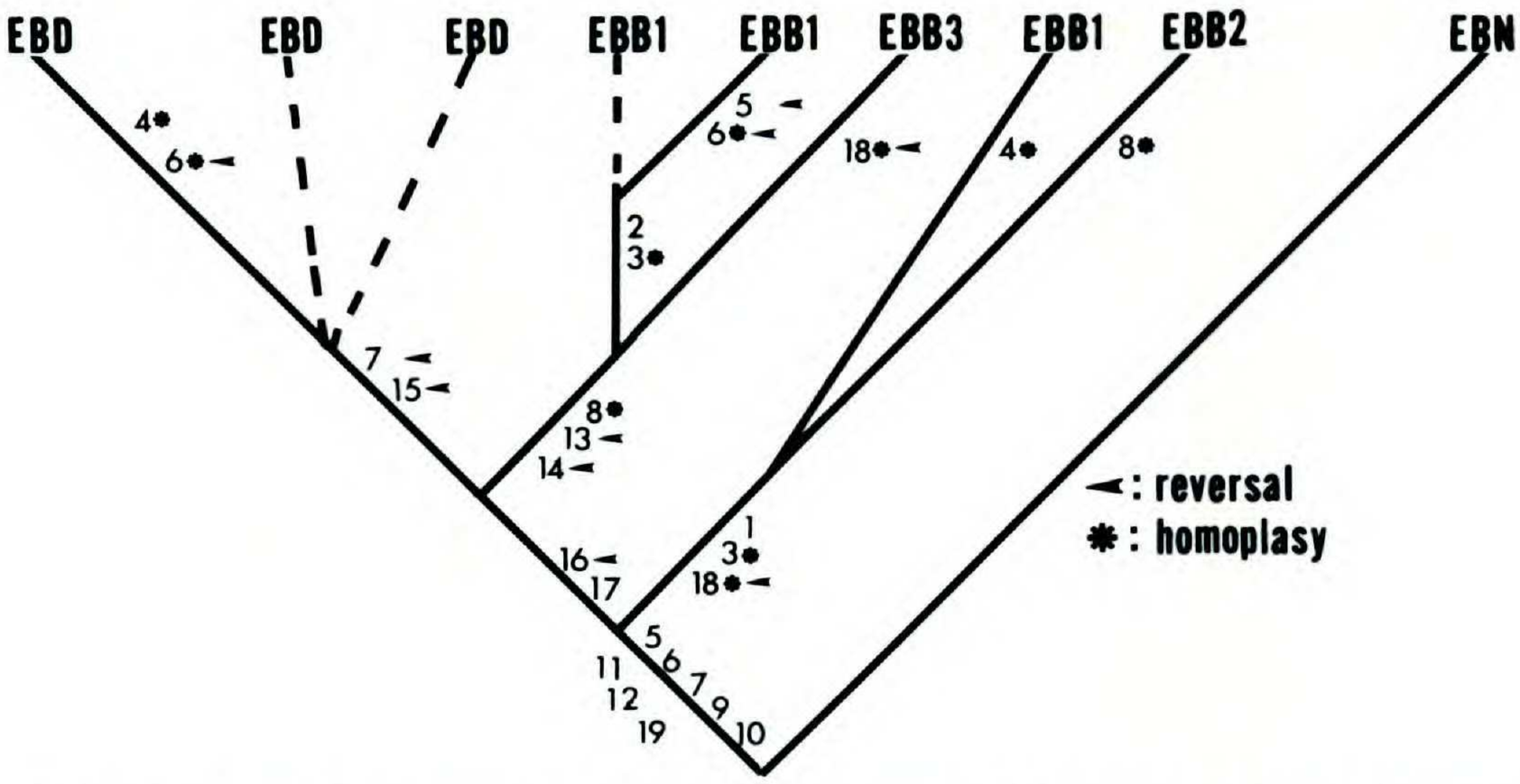


FIGURE 16. Cladogram based on isozyme phenotypes of tetraploid *Eucharis*. Refer to Table 4 for population designations. Broken line indicates zero-length branch.

riety is also an unstable tetraploid. Fifty percent of all chromosome counts of root tip cell mitotic metaphase configurations have $2n = 46$, the typical diploid chromosome number in *Eucharis*. Variety *dressleri* lacks the additive banding patterns or heterozygote phenotypes observed in both loci of AAT in all other populations of *E. bouchei*, a factor, perhaps, of this karyotypic instability. Additive enzyme banding patterns have been observed in a number of tetraploid taxa of *Gossypium* (Cherry et al., 1972), *Nicotiana* (Reddy & Garber, 1971; Sheen, 1972; Smith et al., 1970), *Triticum aestivum* (Hart, 1970, 1979; Jaaska, 1978; Torres & Hart, 1976), and *Stephanomeria* (Gottlieb, 1973), and are usually interpreted as indicative of allopolyploid origins (Crawford, 1983; Gottlieb, 1983; Soltis & Rieseberg, 1986). Pollen stainability of var. *dressleri* is 100% with Alexander's (1969) stain, suggesting that gamete formation is not impaired by the chromosome number instability. Nonetheless, I have not successfully crossed this variety with El Valle populations of var. *bouchei*.

The rare Colombian tetraploid *E. bonplandii* also lacks either heterozygote or additive banding patterns for AAT. This may indicate an autopolyploid origin for this species (Crawford, 1985; Soltis & Rieseberg, 1986), or at least a lower degree of heterozygosity than in *E. bouchei*. The rarity of this species, in relative contrast to *E. bouchei*, fits Stebbins's (1980) model of the "unsuccessful" autopolyploid. The difficulty in obtaining successful meiotic figures from bulbs of *Eucharis* (microsporogenesis occurs completely inside the bulb) blocks investigation of this question. *Eucharis bonplandii* exhibits large distance on the basis of isozyme phenotypes from all individuals of *E. bouchei*. The question of whether these two species represent a monophyletic group on the basis of their tetraploid origin is not conclusive.

Increased heterozygosity is an expected consequence of allopolyploidy (Crawford, 1983, 1985; Gottlieb, 1981a; Soltis & Rieseberg, 1986). A certain degree of fixed heterozygosity would also be expected in an allopolyploid (Gottlieb, 1981a; Soltis & Rieseberg, 1986), due to the presence of two genomes in the allotetraploid. Although it is premature to assess the degree of heterozygosity present in tetraploid *Eucharis*, their morphological diversity and isozyme polymorphisms indicate that it may be high.

There is insufficient information on the breeding system and pollination biology of *Eucharis* to support more than ad hoc hypotheses of the origin of most species in the genus. The characteristically small population sizes that are encountered throughout its range may indicate that founder effects (Mayr, 1954; Templeton, 1980a, 1980b) have played an important role in the movement of *E. bouchei* across the Isthmus of Panama, with subsequent isolation restricting gene flow between localized populations. The putatively allotetraploid genotype and karyotypic polymorphy of *E. bouchei* would favor the "hybrid recombination" type of "genetic transilience," a mode of speciation hypothesized by Templeton (1980a, 1980b). This is consistent with the distribution of *E. bouchei*, most populations of which are geographically isolated from each other (Meerow, 1986). Additional support comes from the morphological novelties expressed

within each geographical variety (or race, in the case of var. *bouchei*). Templeton's model of speciation requires a period of successful inbreeding after the initial founding event. Though *Eucharis* are primarily out-crossing, evidence from greenhouse studies indicates only partial self-incompatibility (Meerow, 1986). At least one Amazonian species of subg. *Eucharis* [*E. castelanaena* (Baillon) Macbride] is autogamous (Meerow, 1986).

EUCHARIS IN CENTRAL AMERICA

Gentry (1982) suggested that two major opportunities, widely spaced in time, existed for floristic interchange between Central and South America. The first, occurring during the Late Cretaceous, was limited to a series of volcanic islands (the proto-Antilles; Dengo, 1975; Lillegraven et al., 1979). The degree to which this island arc remained above water is unknown. At the beginning of the Tertiary, however, this link between the continents was disrupted as the proto-Antilles began a northward displacement. It was not until the late Tertiary that the second opportunity for floristic interchange began to coalesce, as formation of the Central American trench and new volcanic activity gave rise to a new series of islands. These islands eventually formed lower Central America, with a land bridge across the Isthmus of Panama firmly established in the Pliocene, only ca. 3 million years ago (Keigwin, 1978; Marshall et al., 1982). Gentry (1982) concluded that only very well-established Cretaceous taxa would have been able to take advantage of the earlier connection via island-hopping. Entries into Central America dating from this earlier connection would be expected to show strong taxonomic differentiation in Central America. Gentry (1982) cited tribe Crescentieae of the Bignoniaceae as a putative example of early colonization of Central America by island-hopping, followed by taxonomic differentiation. On the contrary, any migration dating from the Pliocene or Pleistocene would not be expected to show much differentiation at the specific or at the generic level. I have characterized the *Eucharis bouchei* complex as a semispecies complex of geographically isolated races or varieties not yet strongly differentiated. Patterns of isozyme variation, chromosome cytology, and morphological variation in this group suggest that entry of *Eucharis* into Central America was fairly recent.

The species of subg. *Eucharis* geographically closest to *E. bouchei* is *E. bonplandii,* a rare species of central Colombia, and also tetraploid. It is inconclusive whether these two species represent a monophyletic tetraploid group. Nonetheless, the congruence of phytogeography with chromosome number in these two species suggests that this may indeed be the case. It is tempting to wonder if tetraploid *Eucharis* were at one time more common in northern Colombia, and if *E. bouchei* and *E. bonplandii* represent the remnant populations of a once more widespread, ancestral tetraploid complex. Prance's (1982) most recent distribution of Pleistocene refugia based on phytogeographic patterns includes both a Río Magdalena refuge in northern Colombia (most collections of *E. bonplandii* are from the Río Magdalena valley south of Prance's proposed refuge), and a Darién refuge in southwestern Panama. *Eucharis bouchei* var. *darienensis* is most common in the area of the Darién refuge and is putatively the least derived variety of the species. The absence of collections of *Eucharis* subg. *Eucharis* from northern Colombia is something of a mystery but may indicate that extinction of intervening populations between *E. bonplandii* and *E. bouchei* was widespread in the recent geological past.

CONCLUSIONS

The Central American *E. bouchei* complex is a tetraploid, putatively alloploid, possibly highly heterozygous, semispecies complex (sensu Grant, 1981) still actively evolving. Discrete patterns of isozyme divergence have not yet solidified between morphologically distinct and geographically isolated populations of *E. bouchei* var. *bouchei.* Founder effects and geographic isolation probably were, and still are, important forces influencing the continued evolution of *E. bouchei.* In one case (*E. bouchei* var. *dressleri*) sympatric divergence may be in process.

The unprecedented degree of variation in *E. bouchei* is thus likely the result of two main factors: (1) tetraploidy, accompanied or followed by structural rearrangement of chromosomes, and (2) a geologically recent colonization of Central America by this primarily northern Andean and Amazonian genus. The wide variation present in *E. bouchei* likely represents the segregating phenotypes of a richly diverse genetic base. On the basis of known distributions, it appears that substantial geographic barriers exist between groups

of populations, probably restricting gene flow between them. Left undisturbed, as is *not* the case in the Neotropics today, these aggregates could conceivably one day each justify specific recognition.

The fact that *E. bonplandii* is the northernmost species of *Eucharis* subg. *Eucharis* in South America, and is also tetraploid, lends at least circumstantial credence to the hypothesis that *E. bouchei* and *E. bonplandii* diverged from a common tetraploid ancestor. The rare occurrence of polyploidy in *Eucharis* strengthens this possibility.

Stebbins (1985), in a recent review of polyploidy, found a correlation between high frequency of polyploidy and patchy geographical (or ecological) distributions, coupled with the occurrence of secondary contact between these differentiated populations. Levin (1983) discussed how chromosome doubling may " 'propel' a population into a new adaptive sphere." Though *E. bouchei* does not exhibit any noticeably novel ecological adaptations, its success in colonizing the Isthmus of Panama may have been aided by its polyploid-related genetic diversity. The heterogeneous isozyme patterns characteristic of El Valle var. *bouchei* may indicate either multiple ancestry for this population or that secondary contact has occurred between it and populations in Colón or Panama provinces to the east.

Eucharis bouchei offers an excellent opportunity for detailed study of the evolution of a tropical rainforest organism. Future work should seek to quantify in greater detail the genetic variation present within and among populations of this actively evolving complex. Meiotic pairing figures from dissection of bulbs may help confirm the nature of the polyploid origins of this species.

Paleotropical genera of "infrafamily" Pancratioidinae characteristically have $2n = 22$ or 20 chromosomes (Ponnamma, 1978; Zaman & Chakraborty, 1974), while almost all neotropical genera have $2n = 46$ (Di Fulvio, 1973; Flory, 1977; Meerow, 1987a, 1987b; Williams, 1981). The latter number is likely derived through fragmentation or duplication of a single chromosome, followed by doubling of the genome (Lakshmi, 1978; Sato, 1938). Increased heterozygosity may therefore have accompanied a tetraploid origin of the neotropical tribes of the Pancratioidinae from an ancestor with $2n = 22$ [the somatic number characteristic of *Pancratium* L., the largest paleotropical genus of the subfamily (Ponnamma, 1978)]. The high generic diversity of neotropical pancratioids (ca. 15 genera) in comparison with the paleotropical taxa (4 genera) itself may be partially a consequence of greater genetic variability. Comparative analysis of isozyme phenotypes between paleotropical and neotropical genera is planned and may provide insight into the evolution of the Pancratioidinae.

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